Hot Projects

Center for Plant Health Science and Technology
Molecular Diagnostics & Biotechnology
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National Crop Biosecurity Center: The development of a National Crop Biosecurity Center

(NCBC) has been initiated to enhance the ability of APHIS to respond to and manage intentional and unintentional introductions of plant diseases and pests. The mission of the Center is to establish a holistic, integrated system to coordinate agency plant biosecurity and emergency response efforts by addressing elements contained in HSPD-9 and the historic mission of APHIS. It will also expand capabilities for diagnostics of plant pathogens, invasive arthropods, nematodes, weeds, and mollusks; facilitate deployment of an effective laboratory certification program with the prerequisite quality assurance and quality control systems in accordance with national and international standards; and serve as the main PPQ laboratory training center.

National Plant Pathogen Laboratory Accreditation Program and the ICLN: The development of

programs to accredit labs for performing PPQ diagnostic tests is currently underway, as the need for this program is being realized by APHIS divisions and many stakeholders. Ongoing processes of protocol validation and laboratory training will increase, as will overall Quality Assurance and proficiency. A provisional approval program for external laboratories to perform APHIS PPQ diagnostic tests for *Phytophthora ramorum* has already been established and is being used. Additionally, a nationwide Integrated Consortium of Laboratory Networks (ICLN) is in development to support the delivery of timely, high quality, and interpretable results through inter-network communication and information sharing, resource optimization, resource coordination, and strategic planning. The purpose of the ICLN is to establish a U.S. homeland security infrastructure with a coordinated and operational system of laboratory networks that provide timely, high quality, and interpretable results for early detection and effective consequence management of acts of terrorism and other events requiring an integrated laboratory response. The ICLN cuts across all Federal agencies, including USDA, DOE, DOD, HHS, FDA, CDC, and others. APHIS PPQ is a full member of the ICLN as is the National Plant Diagnostic Network (under the auspices of USDA CSREES).

Enhanced Diagnostic Systems: New diagnostic tools are being deployed to counter the increasingly complex

challenge to achieve refined detection and identification of pests to fulfill the historic mission of APHIS in the safeguarding of American agriculture. Use of molecular methods allows rapid, sensitive, and specific identifications that previously could not be realized. In addition, there is a constant discovery and evaluation of new technologies that can be adapted to serve APHIS.

***Phytophthora ramorum* National Program and new, improved diagnostic tests:** In the scientific

support for the *P. ramorum* national program, a major focus is development of improved diagnostic systems. A real time PCR-based assay, originally developed at the Central Science Laboratory (York, UK) is in the final stages of validation at the National Plant Germplasm and Biotechnology Laboratory (Beltsville, MD), and will be implemented for the US SOD program in the near future. Additionally, a multi-laboratory, multi-national effort is underway to identify the next molecular diagnostic test to be validated for detection of *P. ramorum*.

Water testing methods for detection of *Ralstonia solanacearum*: There is a need for an accurate, large

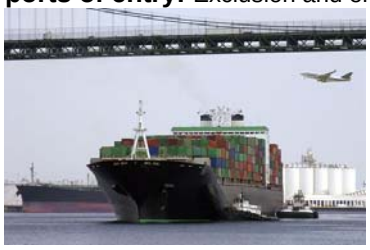
scale, nondestructive method to sample production facilities and consistently detect *R. solanacearum* race 3 biovar 2. The protocol developed from this work will deliver a nondestructive and accurate method to the industry and PPQ for use in off-shore and U.S. facilities. This new protocol could save the geranium industry millions of dollars in lost revenues, and APHIS millions in inspections, detection, and compensation.

Implementation of rapid, on-site detection of citrus canker: This project will result in the rapid identification

of citrus canker in the field and differentiation of strains using simple, sample preparation methods and a real-time PCR assay. Also, a mobile diagnostic laboratory that will allow for proof-of-concept of real-time PCR detection in the field has been constructed. APHIS will validate the performance of field inspector visual identification of canker by having inspectors swab putative canker lesions they identify to test using the real-time PCR.

Adaptation of improved PCR for in-field testing for *Plum pox virus*: Use of certain regions of the *Plum pox virus* genome for PCR detection is the most accurate way to identify the virus in host tissue. An immunocapture-reverse transcripatase-PCR method using conventional PCR was developed by Levy and others in the early 90s. To improve the through-put for survey testing, this test is being adapted to real-time PCR in both the mobile and field-hardened PCR machine from Idaho Technologies and the mobile PCR machine from Cepheid. This technology will be adapted to the field for testing in a mobile diagnostic trailer for use at the farm gate.

Molecular diagnostic techniques that identify foreign sources of fruit fly pest introductions and rapid, molecular identification technology for thrips and immature fruit fly species intercepted at ports of entry: Exclusion and eradication efforts for economically significant pests are frequently dependent upon knowledge of colonization patterns. Determining routes of introduction and levels of infestation are key issues surrounding the control of many unwanted, intercepted pests. Molecular technologies are a proven technology for assessing the level of risk and infection pathway presented by these pests. One goal is to adapt and develop molecular diagnostic tools for rapid identification of intercepted, immature fruit fly species of economic importance. A similar project is aimed at economically important (but very difficult to identify) thrips speces. One outcome will be high through-put, rapid diagnostic capability at Plant Inspection Stations. Another area of emphasis is aimed at sequence-specific, mitochondrial DNA variation in *Anastrepha ludens* to use as a means for identifying the origins of intercepted Mexican fruit fly.



Select Agents: As defined by the Agricultural Bioterrorism Protection Act of 2002, the select agents include 8 plant pathogens which have the potential to pose a severe threat to plant health or plant products. MDB staff are the CPHST points of contact on issues regarding agricultural bioterrorism and are developing an informational matrix that will provide rapid and up-to-date-information on world scientific expertise, diagnostics, risk analysis, mitigation, and other biological information on the select agents.

Implement development of genetically modified insects for biological control: This is a unique project spearheaded by APHIS and driven by the need for new technologies to fight devastating insect pest infestations. Involving APHIS, industry partners, and academic institutions, this program will enhance the ability to use Sterile Insect Technology (SIT) to control or even eradicate infestations. In addition to overcoming the scientific challenges of this endeavor, APHIS is also involved in the process of deregulation and deployment of this technology, including convening a science panel to provide information needed to achieve these goals.